Development of a Physiologically-Based Pharmacokinetic Model for Preterm Neonates:

Evaluation with TDM Data of Amikacin and Paracetamol

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1. Abstract

Among pediatric patients, preterm neonates and newborns are the most vulnerable subpopulation. Rapid developmental changes of physiological factors affecting the pharmacokinetics of drug substances in newborns require extreme care in dose and dose regimen decisions. These decisions could be supported by *in silico* methods such as physiologically-based pharmacokinetic (PBPK) modeling. In a comprehensive literature search, the physiological information of preterm neonates that is required to establish a PBPK model has been summarized and implemented into the database of a generic PBPK software. Physiological parameters include the organ weights and blood flow rates, tissue composition, as well as ontogeny information about metabolic and elimination processes in the liver and kidney. The aim of this work is to evaluate the model's accuracy in predicting the pharmacokinetics following intravenous administration of two model drugs with distinct physicochemical properties and elimination pathways based on therapeutic drug monitoring (TDM) data. To this end, PBPK models of amikacin and paracetamol have been set up to predict their plasma levels in preterm neonates. Predicted plasma concentration-time profiles were compared to experimentally obtained TDM data. For both drugs, plasma concentration-time profiles following single and multiple dosing were appropriately predicted for a large range gestational and postnatal ages. In summary, PBPK simulations in preterm neonates appear feasible and might become a useful tool in the future to support dosing decisions in this special patient population.

Keywords: physiology, preterm, newborn, maturation, pharmacokinetic, amikacin, paracetamol

2. Introduction

Preterm neonates are the most vulnerable subpopulation in pediatrics. Even in Western European countries, the United States of America and Japan preterm birth is still associated with a high risk of morbidity and mortality. Pharmacological interventions in this patient group are frequent but impeded by the fact that the majority of drugs used have not been properly tested and licensed for pediatric application. It has been estimated that up to 93% of neonates in intensive care units (ICU) receive at least one unlicensed medicine during their stay in an ICU [1-3] or a medication in a way not covered by the drug label. This unlicensed or off-label use of medicines in children is associated with an increased risk of serious adverse drug events and fatalities [1-3]. Legislation providing voluntary or mandatory mechanism to conduct clinical studies in pediatric patients is in place in Europe and the US [4, 5]. Although pediatric regulation resulted in general in an increase in clinical trials involving children and label extensions towards use in pediatrics [5], the number of drugs studied in newborns, and in particular in preterm neonates, has remained very small [6]. Because the pharmacokinetics of drugs usually significantly differ between preterm and term neonates, infants and adults, dose and dosing regimen decisions can be extremely difficult for pediatricians and are usually derived from adult regimens relying on heuristics such as linear or allometric extrapolations based on body weight only. Thus, children and in particular preterm neonates undergoing pharmacotherapy could greatly benefit from tools and techniques that support the pediatrician to find a safe and efficient dose or dosing scheme tailored to the individual patient [7, 8].

Due to the ethical concerns and difficulties associated with clinical trials in children and particularly in term and preterm neonates, additional techniques and tools for supporting dose and dosing regimen decisions in pediatrics are highly desired. One of the most promising tools in this context is physiologically-based pharmacokinetic (PBPK) modeling. This technique has become more and more accepted within the pharmaceutical industry and several successful applications have already been published [9-13]. With the help of PBPK modeling, the absorption, distribution, metabolism and excretion (ADME) of drugs in children of various age groups can be predicted based on models established in adults. Such predictions can help to make PK studies in children safer, easier and more reliable.

The aim of this study is to develop a generic PBPK model for preterm neonates. In the first step, the parameters relevant for PBPK modeling that include anatomical and physiological information such as organ weights, blood flow rates as well as maturation information of hepatic enzymes and kidney functions are collected from the literature and implemented into a database. Neonates aged from 40 weeks down to preterms with a gestational age (GA) of 24 weeks were targeted in our literature search. Although rare survivors have been documented as early as 22 weeks of gestational age GA, 24 weeks of GA is generally referred to be the limit of viability at which the newborn has a 50% chance of survival [14]. This typically represents the cutoff point when pediatricians will use intensive medical intervention to save the preterm's life because survival rates are extremely low for babies born earlier. In the second step, the predictivity of the model is assessed based on therapeutic drug monitoring data (TDM) of two drugs, amikacin and paracetamol, in preterm neonates of different GA and postnatal age (PNA). With regard to the administration route, this study focuses on intravenous (IV) drug delivery, which is the main route of drug administration in preterm neonates.

3. PBPK Model development

The preterm model will be implemented in a previously developed generic software platform for PBPK modeling (PK-Sim®, Bayer Technology Services GmbH, Leverkusen, Germany). Up to now, PK-Sim® facilitates PK simulations for a variety of laboratory animals (mouse, rat, dog, minipig, and monkey) as well as humans down to term neonates. The model contains 18 organs and tissues, namely arterial and venous blood, adipose tissue, brain, bone, gonads, heart, kidneys, large intestine, liver, lung, muscle, portal vein, pancreas, skin, small intestine, spleen, stomach. The organs are further divided into four sub-compartments. These are the plasma, the red blood cells (which together build the vascular space), the interstitial space and the cellular space. Compound-specific model parameters such as organ/plasma partition coefficients, permeability-coefficients etc. are calculated from physicochemical properties of the compound and from the composition of tissues in terms of water, protein and the different types of lipids [15]. The generic structure of the PBPK model implemented in PK-Sim® is illustrated in Fig. 1.

3.1. Anatomical data

To extend the physiological database containing the relevant parameters for PBPK modeling towards preterm neonates, a comprehensive literature search was performed. The collected physiological parameters including their developmental changes are summarized in Table 1. Relevant parameters considered in the model are body weight and height, the volumes and blood flow rates of the various organs, the tissue composition and the physiology of the gastro-intestinal tract (besides the fact that oral drug administration is only exceptionally performed in preterm neonates [16]). For a closer look to these data see Table 1 and Figure 2.

Standard weights for normal healthy fetuses and thereby weight and size distributions were obtained from intrauterine growth charts (Fig. 2) [17-25]. In the first few days of postnatal life, preterm (as well as term) neonates typically undergo a short phase of weight loss. This weight loss is equivalent to a “dip” in the postnatal growth curve for body weight as evident in Figure 2. The depth and time of occurrence of the weight curves minimum depends on body weight at birth. The absolute and relative loss in body weight is smallest for the very light and, accordingly, the very young preterms. A preterm neonate with a birth weight of 550 g, for example, loses approximately 5 g (or 1% of its body weight), whereas a baby born near term with a birth weight of 2500 g might lose approximately 150 g (or 6% of its body weight) during the first days of postnatal life [26, 27]. Sex differences were not considered in case of preterm neonates with the exception of the volume of the gonads [28, 29]. For the most important organs, age dependent weights or volumes and their growth rates could be retrieved (Table 1 and Fig. 3A). Apparently, organ growth is not solely related to the body weight, but shows a very complex pattern. In particular the relative contribution of fat and muscle tissue to the overall body weight of a preterm changes during fetal and early postnatal development. Muscle, fat, bone and brain represent the largest organs in preterm neonates (Table 1). Several organs show linear growth (Fig. 3A), whereas the adipose tissue grows exponentially during fetal life. These changes affect the distribution volume of drugs and are, thus, important to be considered in drug dosing decisions.

While direct measurements of organ weights were available for brain, lung, heart, liver, spleen, pancreas and kidney, only indirect measurements were available for skin and stomach. For these organs, weight-age relationships could be established on the basis of the reported surface area and the thickness. It has to be kept in mind, however, that the majority of reported data was obtained post mortem during autopsies, which could have influenced the measurements, especially in case

of the skeleton mass, where the tissue was reported to be macerated [30]. Furthermore, all organ weights summarized in this article refer to exsanguinated organs and require a correction for their blood content, as already described by Edginton *et al*. in order to obtain the total organ weight *in vivo* [10].

Developmental changes in organ blood flow rates were only poorly described in the literature, with only measurements of cardiac output [31, 32] and renal blood flow rates [33] being reported. For the PBPK model it was assumed that the organ-specific percentage contributions to total cardiac output in a preterm newborn are identical to those in term neonates [10], an assumption supported by the data presented for renal blood flow by Visser *et al* [33]. Developmental blood flow changes are depicted in Figure 3B. Similar to the organ specific blood flow rates, the postnatal changes in the organ composition are only poorly described in the literature and had to be extrapolated from term newborns [10].

Physiological information about the gastro-intestinal tract is also available to a sufficient extent, at least with respect to tissue weights. Physiological data that is only relevant for the simulation of oral administration such as luminal pH, geometric dimensions and transit times, were included mainly for the reason of completeness. Their relevance for the PBPK model is limited because oral drug administration is only performed in exceptional cases [16].

Because immature function of the eliminating organs in preterm neonates can contribute considerably to the age-dependence and inter-individual variability of pharmacokinetics, prior knowledge about enzyme activities in the liver and maturity of kidneys during fetal life and in the early postnatal period is essential for a predictive PBPK preterm model. Thus, information about liver and kidney maturation were also compiled in dependence of the GA and PNA as additionally described below. The total age range in this report covers the weeks 24 to 40 of GA (second and third trimester fetuses) and the first two years of postnatal life.

3.2. Model for maturation of glomerular filtration

For the simulation of the age-dependence of glomerular filtration rate (GFR), the maturation model presented by Rhodin *et al*. [34] was modified to make use of the more detailed physiological knowledge, in particular the kidney volume of an individual at a given age. Rhodin *et al*. described the GFR of preterm neonates by a sigmoid maturation function of postmenstrual age (PMA) in combination with an allometric size factor with exponent 0.75 relative to an adult individual with standard weight of 70 kg [34]:

$$\mathrm{GFR} = \frac{\mathrm{PMA}^{\mathrm{Hill}}}{\mathrm{TM}_{50}{}^{\mathrm{Hill}} + \mathrm{PMA}^{\mathrm{Hill}}} \bullet \left(\frac{\mathrm{WEIGHT}}{70\ \mathrm{kg}} \right)^{0.75} \bullet \mathrm{GFR}_{\mathrm{mat}} \qquad (1)$$

Here, TM_{50} denotes the maturation half time and GFR_{mat} represents the mature value for GFR in a healthy adult individual weighing 70 kg. The Hill exponent determines the steepness of the sigmoidal maturation curve. As a measure of the neonates weight (WEIGHT), the best model was obtained by the authors when normal fat mass (NFM) was used rather than total body weight (BW) [34].

In the context of a whole-body PBPK model, the influence of size can be directly displayed by the (*a priori* known) volume of the kidney. We therefore replaced the allometric size factor by a linear scaling factor of the kidney volume at a given PMA normalized to the kidney volume of an adult individual with a standard weight of 70 kg (440 mL [35]). In addition, a small fractional offset in the GFR ($fGFR_{premat}$) was introduced that corrects for a tendency to slightly

underestimate the observed median GFR values in preterm infants below approximately 32 weeks of gestation:

$$GFR = \left(\left(\frac{PMA^{Hill}}{TM_{50}{}^{Hill} + PMA^{Hill}} \bullet \left(1 - fGFR_{premat}\right) \right) + fGFR_{premat} \right) \bullet \left(\frac{Volume_{Kidney}}{440\ mL} \right) \bullet\ GFR_{mat} \quad (2)$$

Eq. (3) was used in this study to re-fit the age-dependent GFR data provided in the study by Rhodin *et al*. [34]. The parameter identification was performed using MATLAB's FMINSEARCH routine yielding the following values (mean ± s.d.): Hill = 15.0 ± 0.3, TM_{50} = 44.4 ± 1.0 weeks, $fGFR_{premat}$ = 0.256 (fixed), GFR_{mat} = 117 ± 27 ml/min. The resulting description of GFR vs. age is shown in Fig. 4 in comparison to the median and 90% confidence interval of the data collected by Rhodin *et al*. [34] for a virtual population of preterm neonates (A) and over the first 20 years of life (B). For validation purposes, the results of the modified Rhodin-model were also compared to a second set of GFR maturation data in preterm neonates previously presented by Vieux *et al*. [36] which showed overall correspondence.

3.3. Model for liver maturation

The liver is the main organ involved in drug metabolism. Besides the liver size, metabolic phase I or II reactions depend on the activity and amount of drug metabolizing enzymes (DME). Infants have different capacities to metabolize drugs, so that some substances might produce either lower or higher plasma concentrations than expected. The differences in enzyme activity can alter the production of intermediate metabolites. DME ontogeny has been recently reviewed by Hines *et al*. [37]. A summary of the data collected by the author is given in Table 2.

Apparently, there are three different groups of DME. Enzymes belonging to the first group are expressed at their highest level during the first trimester. These enzymes remain on this high level of expression or decrease during gestation until they get silenced with birth. Cytochrome P450 (CYP) 3A7, flavin monooxygenase (FMO) 1, sulfotransferase (SULT) 1E1 and perhaps glutathione S-transferase (GST) 3 belong to this group. Enzymes revealing the expression patterns of the second category, *e.g.* CYP3A5, SULT1A1 and UDP-glucuronosyltransferase (UGT) 1A3, show relatively constant levels throughout life. Enzymes that are not or weakly expressed during gestation and that show an increase of expression during late gestation or postnatally belong to the third group of enzymes. The majority of DMEs obeys this expression pattern and for example includes alcohol dehydrogenase (ADH), CYP2C9, CYP3A4, and UGT2B17. The increase in enzyme expression is most often observed within the first or second postnatal year [37]. Up to now, the knowledge about the ontogeny of enzymes still increases and gives further insight into drug metabolism in developing infants.

In addition to drug metabolism via enzymes, drugs can be excreted via bile. Development from progenitor cells into a differentiated organ, in which bile secretion can be observed, occurs at around 12 weeks of GA [38]. Therefore, the biliary excretion in the model is expected to be the same as in term neonates [39].

The preterm model was extended by integrating the information about hepatic enzyme ontogeny in fetuses and preterm neonates as referred in Table 2. The enzyme ontogeny necessary for the prediction of paracetamol PK is described in more detail below. Elimination of paracetamol occurs primarily by hepatic metabolism (95% of total body clearance). Three enzymes are mainly responsible for paracetamol hepatic clearance. Metabolization occurs via CYP2E1, via SULT1A1

and via UGT1A6. Compared to adults, enzyme activity in preterm neonates and fetuses is reduced to 0-10% for CYP2E1 as this enzyme was not detected in fetuses, but arises postnatally. SULT1A1 is reduced during all fetal stages to 62% and UGT1A6 is reduced to 1-10% of adult enzyme activity [40-42]. All three enzymes thereby belong to the third group of enzymes reviewed in Table 2, that is, their enzyme activities increase postnatally.

3.4. Model evaluation: Amikacin

Amikacin is an aminoglycoside antibiotic used for short-term treatment of serious infections (due to multidrug resistant Gram negative bacteria such as *Pseudomonas species* [43]). Since amikacin is excreted primarily by GFR [44, 45] the clearance of amikacin should be predictable solely based on knowledge about kidney maturation and, accordingly, developmental changes in GFR.
For the evaluation of the newly established PBPK model for preterm neonates, the pharmacokinetics of amikacin were predicted based on an amikacin model previously established for adults [46]. The drug related parameters described in Table 3 were kept as defined in the adult model, whereas age-dependent parameters including anatomy and physiology as well as the developmental changes in drug clearance were adapted using the parameter database described in Table 1 and Fig. 2 and 3.
Simulated data was compared to experimentally obtained amikacin levels [47]. In the experimental study, 701 preterm and term neonates (GA 24 to 41 weeks) were given amikacin according to a GA-based dosing chart during their first days of life. This chart was developed in 2002 by Langhendries *et al*. [48]. Recommended doses were: GA < 28 weeks: 20 mg/kg/42 hours, GA 28-30 weeks: 20 mg/kg/36 hours, GA 31-33 weeks: 18.5 mg/kg/30 hours, GA 34-37 weeks: 17 mg/kg/24 hours, GA > 37 weeks: 15.5 mg/kg/24 hours. Amikacin was given as an IV infusion over 20 minutes. In general, blood samples were collected by arterial line or venous puncture just before (trough value) and 40 minutes after completion of the second IV dose of amikacin [44, 45].
We simulated virtual preterm populations consisting of 500 male and 500 female preterm subjects for each PMA. The GA ranges from 24 to 40 weeks of gestation with postnatal growing steps of one day up to 30 days of PNA. Thus, a total of 480 populations for the span of 16 weeks of GA times 30 days of PNA, each including 1000 individuals, were simulated. The dynamic growth occurring during repeated drug administration as described earlier is also considered in these populations. From these populations, ten virtual individuals with the same PMA at birth and minimal differences in body weight when compared to the patients in the in vivo studies were selected for comparison. For those, the percentage % of predicted data points deviating by a factor of two, three and ten were calculated according to

$$\%_x = n_{factx} / n_{total} \quad (3)$$

The index x indicates the factor of deviation (x = 2, 3, or 10). Here, n_{factx} denotes the number of datapoints that fall within the interval [1/x..x] and n_{total} is the total number of datapoints.

3.5. Model evaluation: Paracetamol

Paracetamol (acetaminophen) is used as an antipyretic and analgesic agent for preterm neonates having painful treatments, or as additional treatment for neonates receiving opioids [49]. In adults, the metabolization via CYP2E1 accounts for about 10%, the metabolism mediated by SULT1A1 accounts for approximately 30-35% and the metabolism via UGT1A6 accounts for 45-55% of the total body clearance, respectively [50].Overall, the age-dependence of paracetamol PK is

governed by hepatic maturation and ontogeny of enzymes involved in its metabolism (see section 3.3). Paracetamol is frequently administered intravenously in the form of its prodrug propacetamol, which is rapidly hydrolyzed to paracetamol by plasma esterases [42]. After IV administration of 2000 mg of propacetamol, the prodrug is hydrolyzed to 1000 mg of paracetamol [3].

Starting point for the paracetamol model for preterms was a previously published paracetamol model for adults and children [51]. The physicochemical properties of paracetamol are summarized in Table 3. For the evaluation of the model predictions, data obtained in 108 neonates were available [41, 42], from which measurements of 106 neonates could be used (two patients were excluded from this analysis because they were older than 40 weeks of GA). In the *in vivo* study, preterm neonates with a PMA of 7 to 43 weeks of gestation and a PNA of one to 76 days were given either single (n=39) or multiple doses (n=69) of 20 mg/kg propacetamol as an infusion over 15 minutes. With respect to age, the virtual populations were the same as for the amikacin study. That is, virtual populations of 500 male and 500 female preterm subjects for each PMA, ranging from 24 to 40 weeks of gestation with growing steps of one day were used. As already described for amikacin, ten virtual individuals with the same PMA at birth and minimal differences in body weight when compared to the patients of the *in vivo* paracetamol study were selected and the mean values from these populations were taken for the individual simulations. For model analysis calculations see section 3.4.

4. Model evaluation results

4.1. Amikacin

Fig. 5 shows the correlation between the predicted and observed amikacin plasma concentrations in preterm neonates (dots), with the solid line indicating the line of identity (solid line). The data are classified by color with respect to PMA ranging from very young preterm infants with a PMA of 24 weeks (blue) to term born neonates with a PMA of 40 weeks (dark red). The results indicate that the predicted plasma concentrations depend on PMA. In particular peak plasma concentrations of predicted plasma amikacin levels rise with smaller gestational age. This leads to the separation of the predicted peak plasma concentrations of amikacin according to gestational ages, as evident from Fig. 5. This separation disappears in the case of the trough concentrations due to the high interindividual variability in renal clearance. This indicates a slightly higher variation in GFR than found in the predicted data, while the opposite was evident in the case of the trough values.

Calculations of the deviations between predicted values and TDM data reveal that 78.4% of all amikacin predictions have a deviation factor of two or smaller. More than 90% of the predictions fall within a deviation factor of three and 98.5% within a deviation factor of ten. Only a slight bias towards lower predicted concentrations might be observed in the higher postmenstrual ages (38-42 weeks of PMA). Most of the underpredicted plasma concentrations can be explained by renal pathologies documented by extremely high serum creatinine levels (SCr), as shown in Fig. 5B. Here, the ratios of predicted/observed plasma concentrations of amikacin vs. experimentally obtained plasma creatinine values are shown. This figure reveals a trend of decreasing plasma creatinine values with rising PMA. Additionally, the plasma creatinine rises with a larger bias towards lower predictions. Most preterms with exceptionally high creatinine values are of higher PMA.

Amikacin pharmacokinetics for the multiple dosing of amikacin are shown in Fig. 6 for three exemplary preterms. The individuals were selected for different gestational ages, multiple dosing and availability of TDM data points. Individuals shown in Fig. 6 are 27, 33 and 36 weeks of PMA. As obvious, after a treatment phase of one week there was a washout period of one week, followed by a second and, if necessary, a third therapy cycle. Overall, the simulated plasma concentration-time curves match the experimentally observed TDM data quite accurately regardless of the gestational or postnatal age.

4.2. Paracetamol

In Fig. 7, the predictions of paracetamol plasma concentrations are plotted against plasma concentrations obtained from TDM data. Again, increasing postmenstrual ages are illustrated by the color of the data points. In case of paracetamol, no tendency towards higher plasma levels with decreasing gestational or PNA could be observed. Overall, the model predicted the paracetamol plasma concentrations of preterm neonates quite accurately: 92.1% of all predicted paracetamol plasma levels were within 2-fold of the observed data, 98.8% of predicted data points fall within a deviation factor of three and 99.6% have a maximum deviation of 10 from the TDM data. No biases, e.g. related to age, were detected. Additionally, individual plasma concentration-time curves of paracetamol are shown (Fig. 8) for three exemplary individuals aged 31, 36 and 40 weeks of GA. The simulated plasma concentration-time curves almost perfectly match the data observed in the clinic.

5. Discussion

Neonates born prematurely often require intensive care treatment including pharmacotherapeutic interventions. In most of the cases, drugs that are administered in the neonatal intensive care units have neither been licensed nor properly studied in this patient group. Unfortunately, preterm responses to drugs differ from those of adults, children and term babies because of pharmacokinetic and pharmacodynamic differences. In the postnatal episode, developmental changes occur at different rates both between individual preterms and when compared to term neonates. For these reasons, the safe and efficient drug treatment of preterm neonates still remains an enormous challenge for the pediatrician. In pharmaceutical development, researchers responsible for the planning of clinical studies in children (often including preterm neonates) have to propose the first dose in children on the basis of clinical data obtained in older patients starting from adults through adolescents down to toddlers and newborn individuals. Both in clinical practice and drug development, a knowledge-based approach considering all available information about physicochemistry of the drug and the state and rate of development of the preterm neonate patients as a function of GA and PNA is highly desirable.

In this study we investigated if whole-body PBPK modeling can potentially serve this need. The PBPK technology has been widely applied to various aspects of drug research and development [52] as well as in environmental risk assessment [53], and its acceptance in the industry and in regulatory agencies – in particular with respect to pediatric applications – is increasing. While several successful application examples in adult humans and children down to term neonates [10, 54] have been published in the literature, preterm neonates have so far not been a subject of physiologically-based simulations. A major hurdle was the availability of the relevant age-dependent anatomical and physiological information that is required to parameterize a PBPK model. This hurdle was overcome by a comprehensive literature search (Fig. 2-3, Table 1-2). The

required data could be gathered and implemented into the database of PK-Sim® allowing a – technically simple – down-scaling of pre-existing human adult PBPK models.
To evaluate the predictivity of the preterm PBPK model, two model drugs were selected based on the (i) the availability of clinical data in preterm neonates and (ii) differences in drug distribution and elimination behavior. While amikacin is a renally cleared antibiotic with relatively low distribution volume (approximately 0.25 – 0.50 L/kg in adults [55]), the analgesic drug paracetamol mainly undergoes hepatic metabolization and distributes to a greater extent to peripheral compartments (distribution volume in adults around 1 L/kg [56]).

The comparison of predicted plasma levels with TDM data obtained in the clinic for both drugs indicated that the physiologically-based model is indeed able to accurately predict the pharmacokinetics in preterm neonates. In case of amikacin, 78.4% of the predictions are within a factor of two or smaller of the TDM data, in case of paracetamol, the accuracy was even better with 92.1% of all predicted data points being within a two-fold range of the observed data. Inter-individual variability in the GFR (as was evident from reported serum creatinine values) was identified as the main cause of the under-predicted amikacin plasma concentrations. This observation implicates that the GFR, which was included based on a modified version of the GFR model previously established by Rhodin *et al*. [34], is not appropriate for some of the preterm patients. This is not surprising because only healthy neonates were considered by Rhodin *et al*., while the TDM studies for amikacin (as well as paracetamol) included hospitalized preterms that were treated for diseases with at least the mentioned drugs. The exceptionally high plasma creatinine values of near term infants included in the amikacin study indicated a possible renal damage and, as a result, a lower GFR has to be expected. The trend towards rising plasma creatinine values for preterm babies with lower PMA can be explained by the fact that these babies were of lower PNA. As such, the plasma levels are affected by the maternal creatinine values. At birth, infants have a plasma creatinine value of maternal circulation which decreases with postnatal age.
These very good predictions of the paracetamol TDM data implicate an appropriate description of the underlying physiological and anatomical data, a correct estimation of the volume of distribution and hepatic metabolism of the drug in preterm patients.
In conclusion, the presented PBPK model is a very promising first step towards a tool that predicts the pharmacokinetic behavior of a drug in preterm neonates. The physiological database contains the relevant data for virtual individuals down to a gestational age of 24 weeks. In the future, the tool might be applied to support dosing decisions and improve the treatment options for the very young ones and, thus, provide helpful information for designers of clinical studies involving preterm neonates as well as caretakers in neonatal units.

6. Conflict of Interest

Kirstin Thelen, Katrin Coboeken, Thomas Gaub, Jörg Lippert and Stefan Willmann are employees of Bayer Technology Services GmbH, the company that owns and commercializes the software platform used for the simulations.

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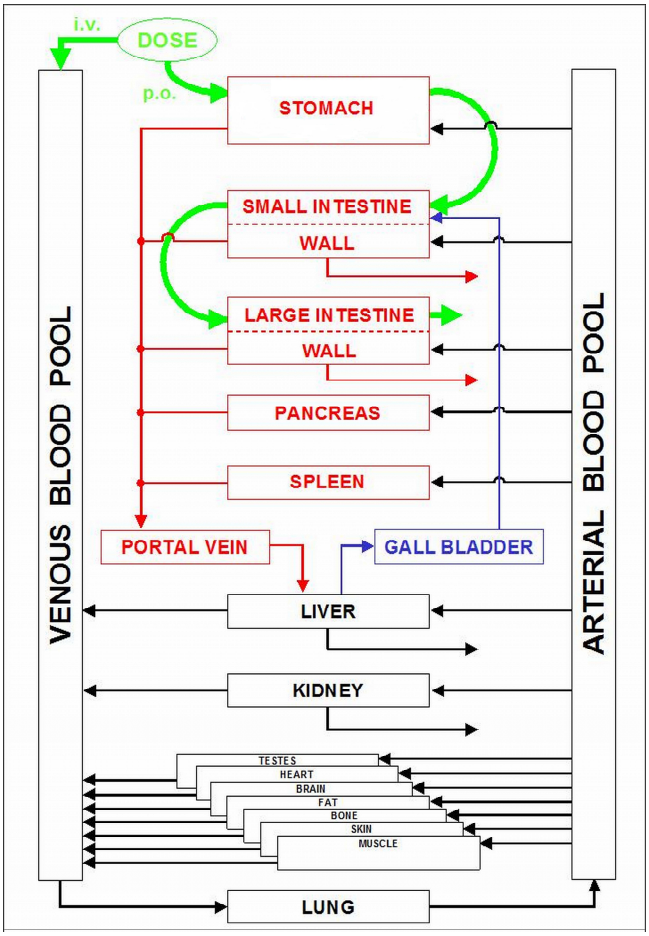
9. Figures



633

634 **Fig. (1).** Structure of the whole simulation model of PK-Sim® with all included
635 organs.

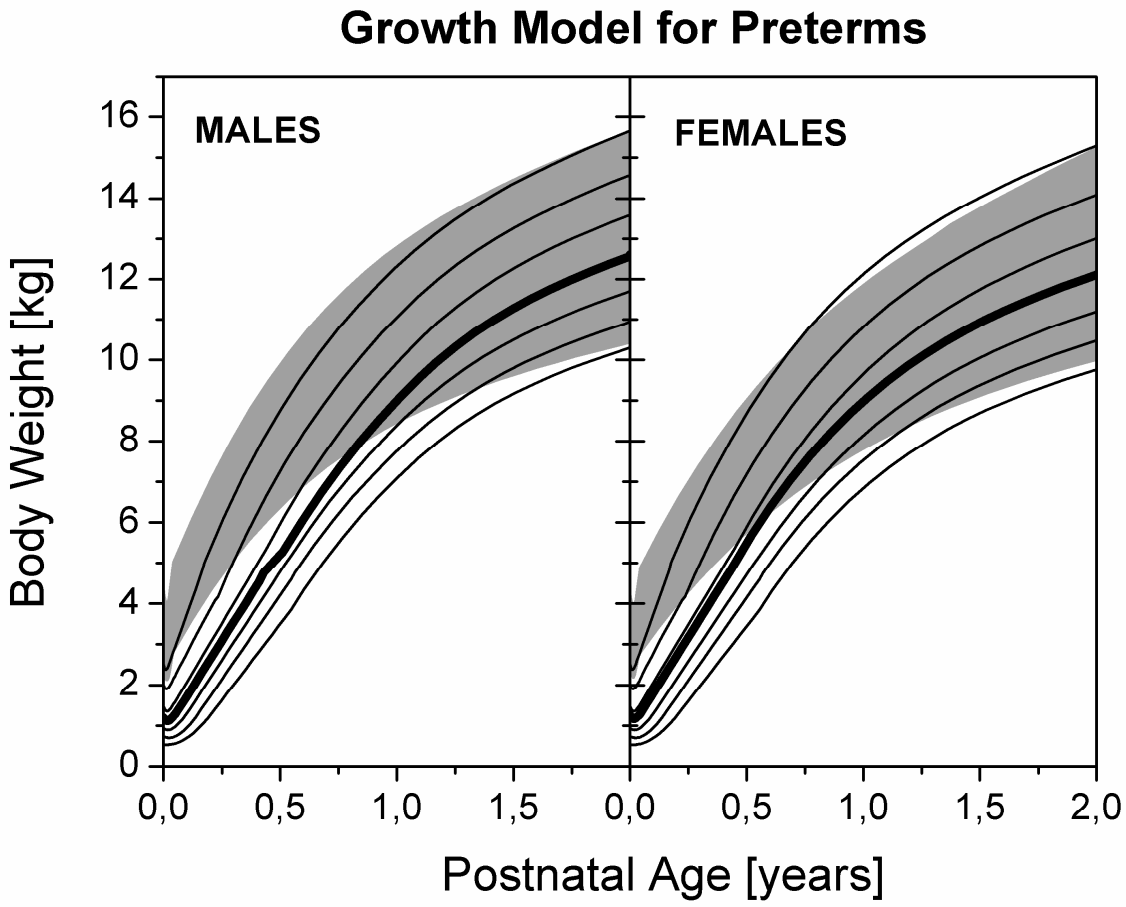
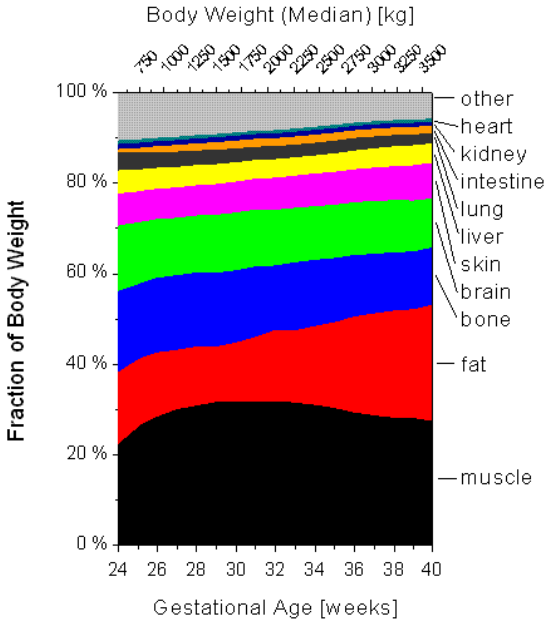


Fig. (2). Postnatal growth curves for preterm infants pooled in different weight classes up to two years (black solid lines) compared to term born infants matched to the growth charts of the "Centers for Disease Control and Prevention" (CDC) (grey-shaded area). Postnatal changes in body weight [kg] are shown.

A)



B)

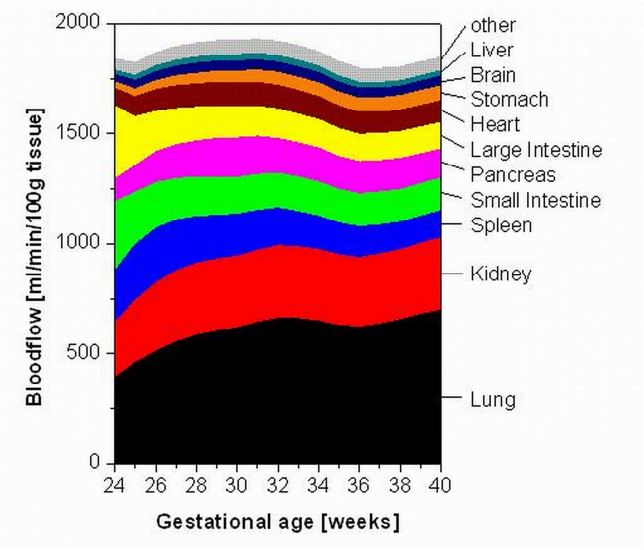


Fig. (3). Physiological data obtained from literature review: **A)** Median organ weights for different organs in preterm infants from 24 to 40 weeks of as percentage of body weight. The median body weight per GA is shown. **B)** Estimation of blood flow for different organs in preterm infants from 24 to 40 weeks of GA in ml/min/kg tissue weight.

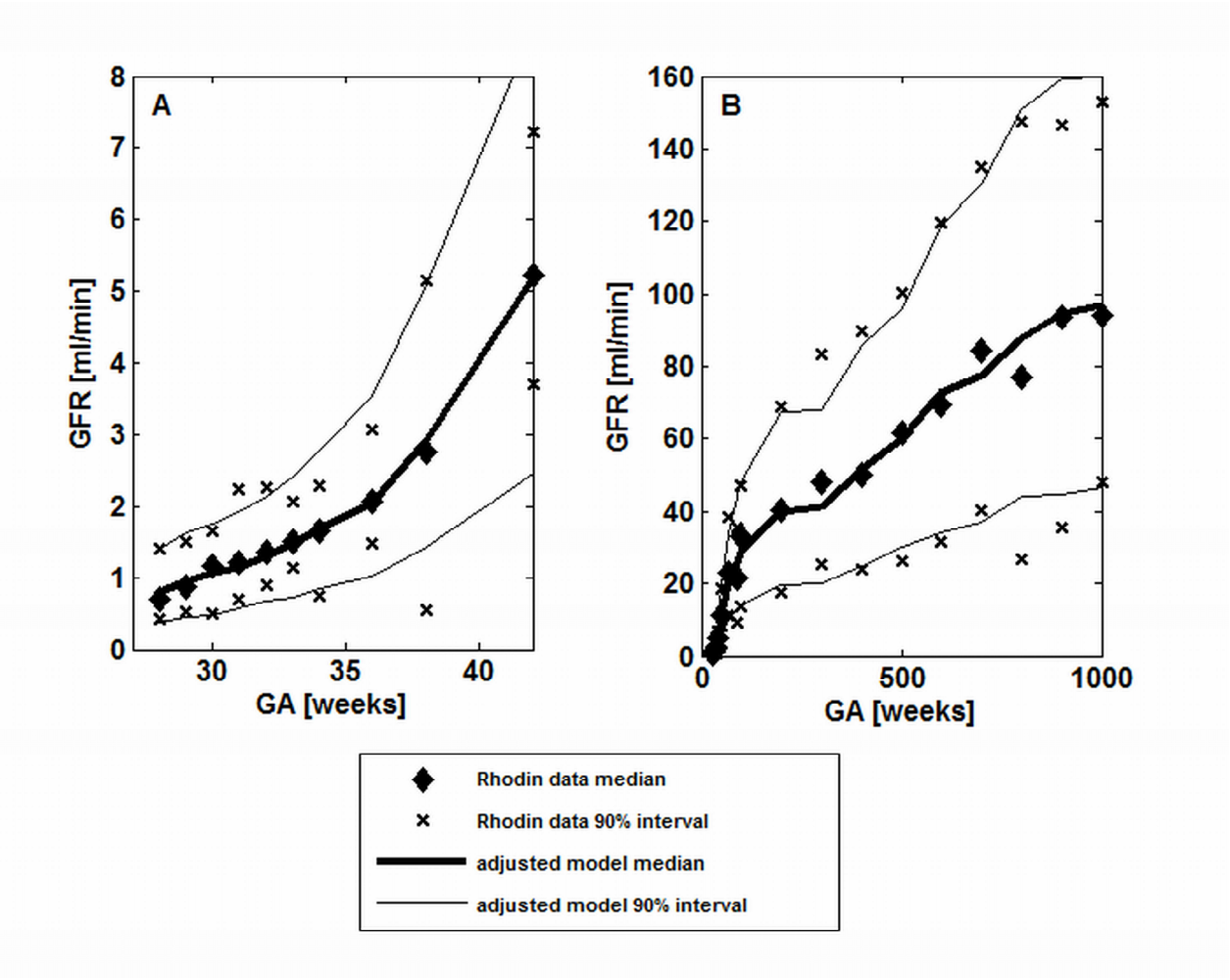
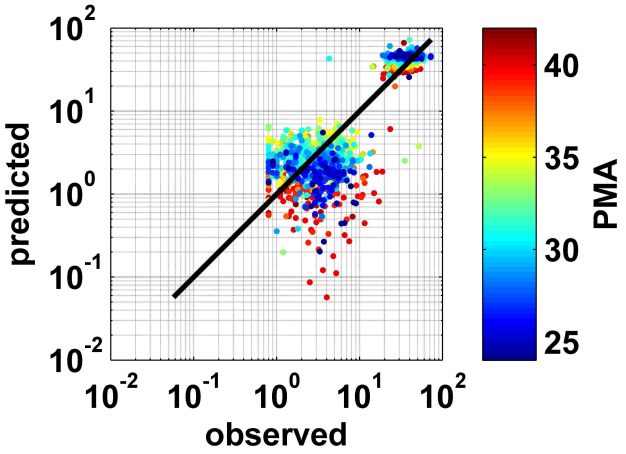


Fig. (4). Representation of changes in GFR (in ml/min) with gestational age (in weeks) in comparison to the data presented by Rhodin *et al*. In both plots the original Rhodin model is indicated as grey line, while our adjusted model is represented by black lines. **(A)** Zoom in to the first weeks of GA **(B)** Development of GFR over the first 20 years of life.

(A)



(B)

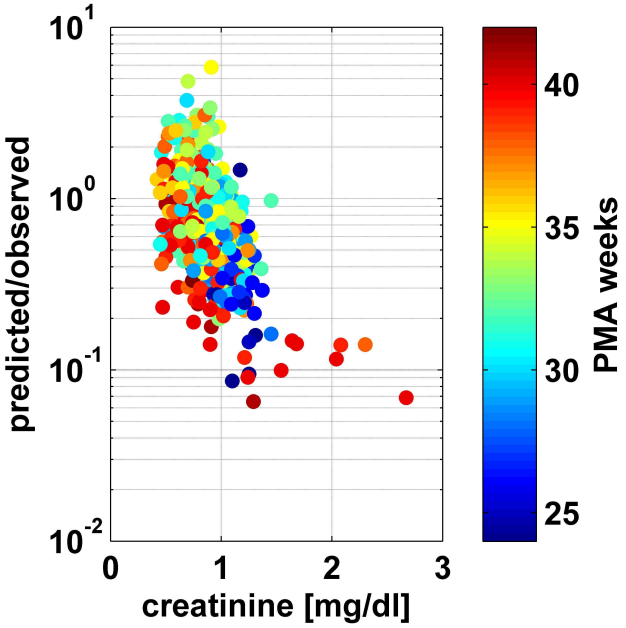
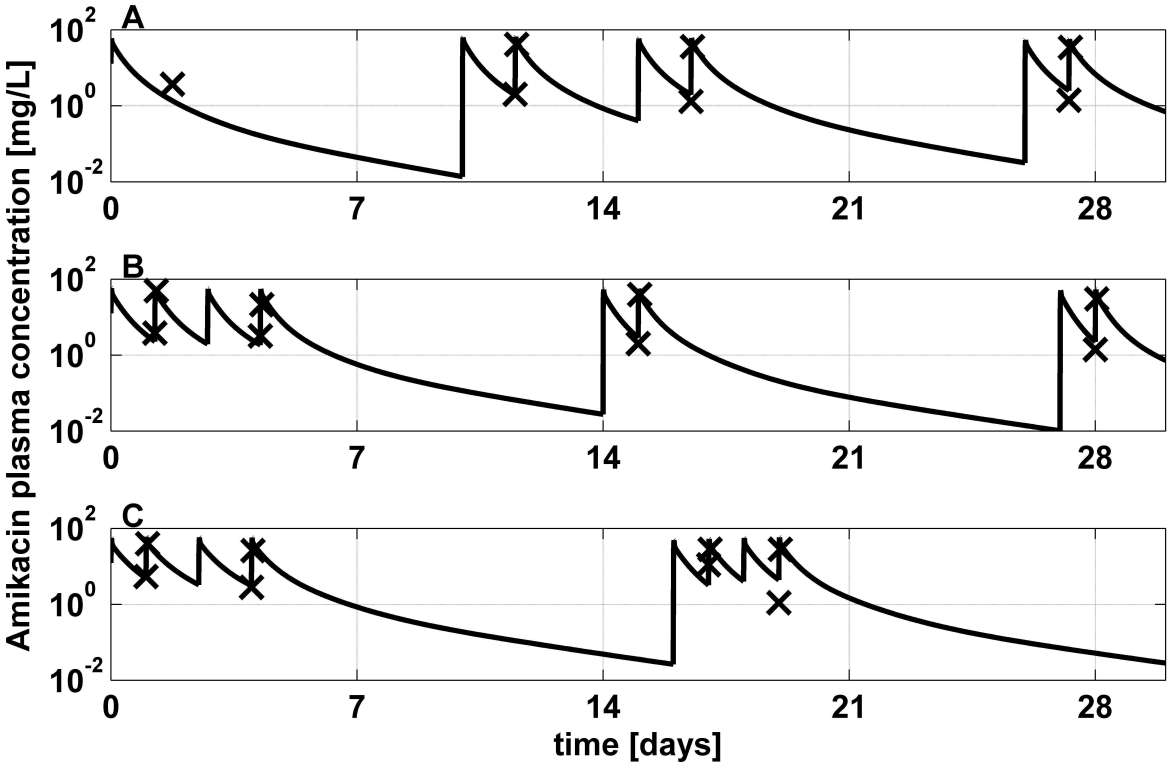


Fig. (5). (A) Comparison of plasma amikacin concentrations of population simulations with amikacin TDM data [44, 45] from preterm neonates at different PMA's **(B)** Changes of creatinine values [mg/dl] with the ratio of predicted vs. observed plasma concentrations (shown in 3A) with PMA.



688

689 **Fig. (6).** Comparison of predicted plasma concentration time curves (solid line) with
690 patient data (x) for multiple doses of amikacin in three representative patients with
691 ages of **(A)** 27 weeks GA, PNA 1 day, **(B)** 33 weeks GA, PNA 1 day, **(C)** 36 weeks
692 GA, PNA 1 day at the day of administration.

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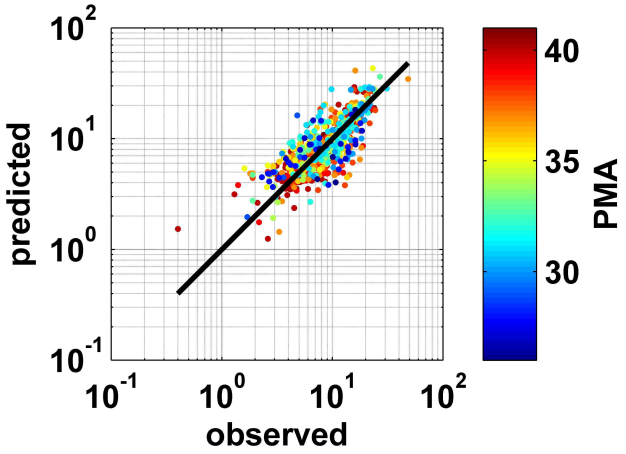
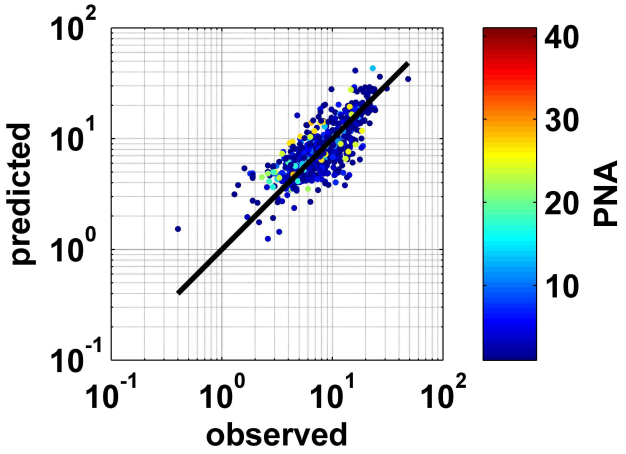
712 **(A)**



713
714 **(B)**



715
716 **Fig. (7).** Comparison of plasma paracetamol concentrations from population
717 simulations with paracetamol TDM data [44, 45] from preterm neonates at **(A)**
718 different PMA's and **(B)** different PNA's.
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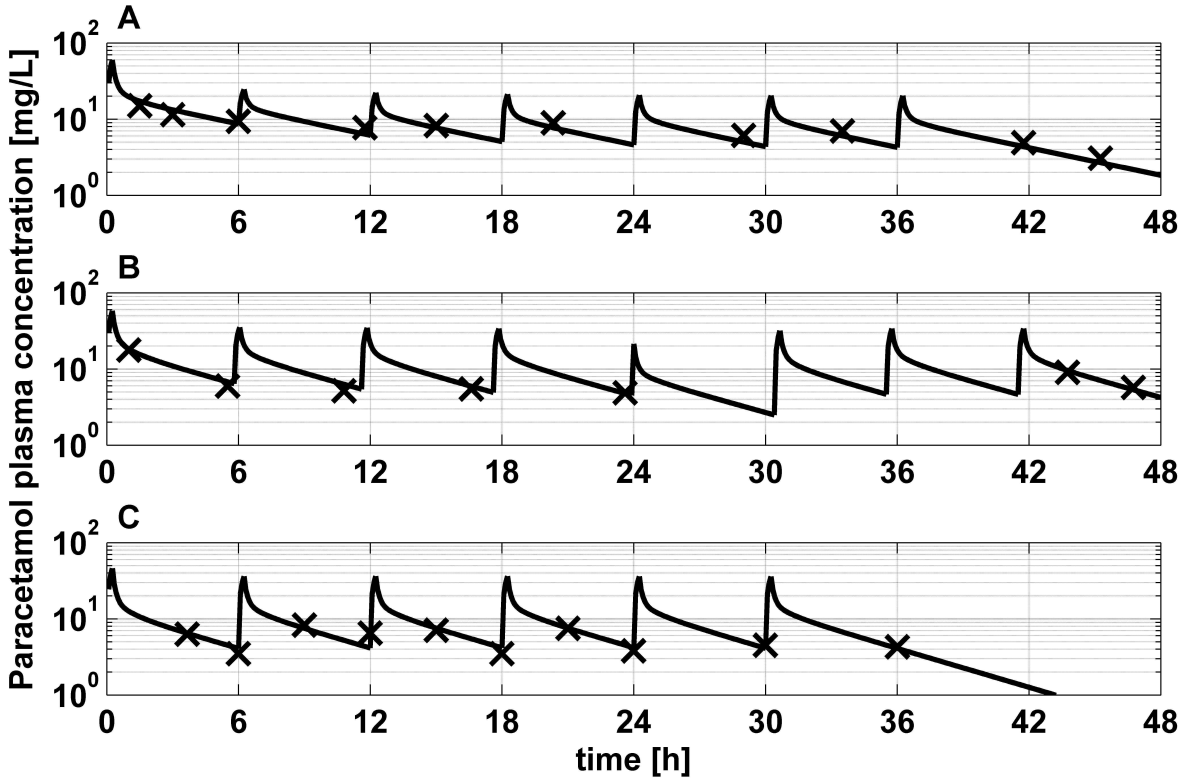

Fig. (8). Comparison of predicted plasma concentration time curves (solid line) with patient data (x) for multiple doses of paracetamol in three different patients at the ages of **(A)** 31 weeks GA, PNA 2 days, **(B)** 36 weeks GA, PNA 17 days GA, **(C)** 40 weeks GA, PNA 4 days at the day of administration.

10. Tables

Table 1: Source, data type and publication date of data about organ weights and organ growth in preterm neonates included in the PBPK model.

Data Set	Year	Used in the model for following Organs	Methods	Population
Archie *et al* [57]	2006	Brain, Heart, Kidney, Liver, Lung, Pancreas, Spleen	Autopsy	Fetal and Neonatal
Ben-Haroush *et al* [58]	2005	Stomach	Sonographic examination	Fetuses 16-42 weeks of GA
Koo *et al* [59]	2000	Adipose tissue	Dual energy X-ray absorptiometry (DXA)	Neonates, 27-42 weeks of GA
Malas *et al* [28]	2001	Gonads (Testis)	Autopsy	Fetuses, 12-40 weeks of

				GA
Malas *et al* [60]	2003	Jejunum, Ileum	Autopsy	Fetuses 10-40 weeks of GA
Malas *et al* [61]	2004	Colon	Autopsy	Fetuses 10-40 weeks of GA
Meban *et al* [62]	1983	Skin	Autopsy	11-42 weeks of GA
Modi *et al* [63]	1990	Muscle	Urinary creatinine	Neonates, 25-34 weeks of GA
Petersen *et al* [64]	1995	Skin	High frequency ultrasound	Newborn infants
Roe [65]	1933	Skin	Autopsy	Fetuses
Shah *et al* [66]	1988	Small, Large Intestine	Autopsy	Fetuses, 20-40 weeks of GA
Smith [67]	2002	Blood volume	Posttransfusional HCT and GA	Preterm infants 21-35 weeks of GA
Struijs *et al* [68]	2009	Intestinal lengths	Autopsy	Infants 25 weeks of GA to 5 years
Sulak *et al* [29]	2004	Gonads (ovary)	Autopsy	Fetuses 9-40 weeks of GA
Touloukian *et al* [69]	1983	Intestinal lengths	Autopsy	19-40 weeks of GA
Trotter *et al* [30]	1969	Skeleton	Autopsy	Fetuses

Table 2: Ontogeny of drug metabolizing enzyme activities in % of adult enzymatic activity for fetuses and preterm infants (based on Hines [70]).

	Early gestation 10-20 weeks of GA	**Mid gestation 21-30 weeks of GA**	**Late gestation 31-40 weeks of GA**	**postnatal**	**Ref.**
Oxidative enzymes					
Alcoholdehydrogenase					
ADH		31%*		45%	Smith *et al*. [71]
Cytochrome P450 system					

CYP1A1		low or no expression*			Shao *et al*. [72]/ Hines *et al*. [37]
CYP1A2		5%*		10%	Alcorn *et al*. [73]
CYP1B1		undetectable*			Bieche *et al*. [74]
CYP2A6		low or no expression*			Gu *et al*. [75]
CYP2A13		undetectable*			Bieche *et al*. [74]
CYP2B6		50%			Croom *et al*. [76]
CYP2C		0%*		3%	Alcorn *et al*. [73]
CYP2C9	0%	4-5%	10%	25%	Koukouritaki *et al*. [77]
CYP2C18		undetectable*			Bieche *et al*. [74]
CYP2C19	0%	1%	10-20%	50%	Koukouritaki *et al*. [77]
CYP2D6	5%	5%	6%	9%	Treluyer *et al*. [78]/ Alcorn *et al*. [73]
CYP2E1	0%	0%	0%	10%	Vieira *et al*. [79]
CYP3A4		3%		13%	Alcorn *et al*. [73]
CYP3A5		is present, no change as function of age*			Stevens *et al*. [80]
CYP3A7		500%*		130%	Alcorn *et al*. [73]/ Stevens *et al*. [80]
FMO system					
FMO1	100%	50%	25%	0%	Koukouritaki *et al*. [81]
FMO3	0%	0%	0%	50%	Koukouritaki *et al*. [81]
Conjugation enzymes					
Epoxide hydrolase					
EPHX1	6%	10%	32%	50%	Omiecinski *et al*. [82]
Glutathione S-transferases					
GSTM (GST1)		22%*		93%	Strange *et al*. [83]
GSTA (GST2)		25%*		62%	Strange *et al*. [83]
GSTP (GST3)		5300%*		2100%	Strange *et al*. [83]
Sulfotransferases					
SULT1A1		62%*			Stanley *et al*. [84]
SULT1A3		340%*			Stanley *et al*. [84]
SULT1E1		106%*			Stanley *et al*. [84]
SULT2A1		26%*			Stanley *et al*. [84]

UDP glucuronosyltransferases			
UGT1A3	30%*		de Wildt *et al.* [85]
UGT1A6	1-10%*		de Wildt *et al.* [85]
UGT2B7	10-20%*		de Wildt *et al.* [85]
UGT2B17	8%*	11%	de Wildt *et al.* [85]

* = values refer to all fetal stages

Table 3: Physicochemical properties of the two model drugs.

Parameter	Amikacin	Paracetamol
Lipophilicity (log MA)	-0.48	0.78
Molecular weight [g/mol]	585.6	151.16
Plasma fraction unbound fu)	1.0	0.82